Impact of the transgene MON 810 in expression of coding and non-coding RNAs in maize

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Introduction

MON 810 transgenic maize is the unique genetically modified (GM) maize approved for cultivation within the European Union.
Prior "omics" studies on MON 810 maize in comparison with non-genetically modified near-isogenic varieties has given a broad picture on the differences between transgenic and non-transgenic maize. microRNAs are small non-coding RNAs known to regulate gene expression of several genes. Here, in these study we aimed to find differences in expression of coding and non-coding genes caused by the presence of the transgene. For that, we have performed microarrays of mRNA and miRNAs from two pairs of maize (transgenic/ isogenic).
Interestingly, our results indicate possible "side effects" associated with the transgene integration into the host genome.

Material and Methods

- Leaves from two GM varieties (Talca and Elba) and their near-isogenic non-GM (Ordino and Beret, respectively), were excised from F_1 maize plants in V7 developmental stage;
- Segregating embryos from F_2 grains obtained from a self-pollinated MON 810 population (Elba) were also excised;
- Total RNA from leaves was extracted using TRIzol Reagent and miRNeasy Plant Mini Kit (Qiagen) was used for embryos.
- Profiles were obtained through GeneChip® Maize Genome Array and GeneChip® miRNA 4.0 Array (Affymetrix);
- Genes were considered differentially expressed when having a two-fold change between transgenic and non-transgenic tissues;
- Confirmation was made by real-time PCR based on SYBR®Green and 18S and ubiquitin as reference genes;
- Target transcripts of differentially regulated miRNAs were predicted through "*psRNA target: A Plant Small RNA target server*".

Results

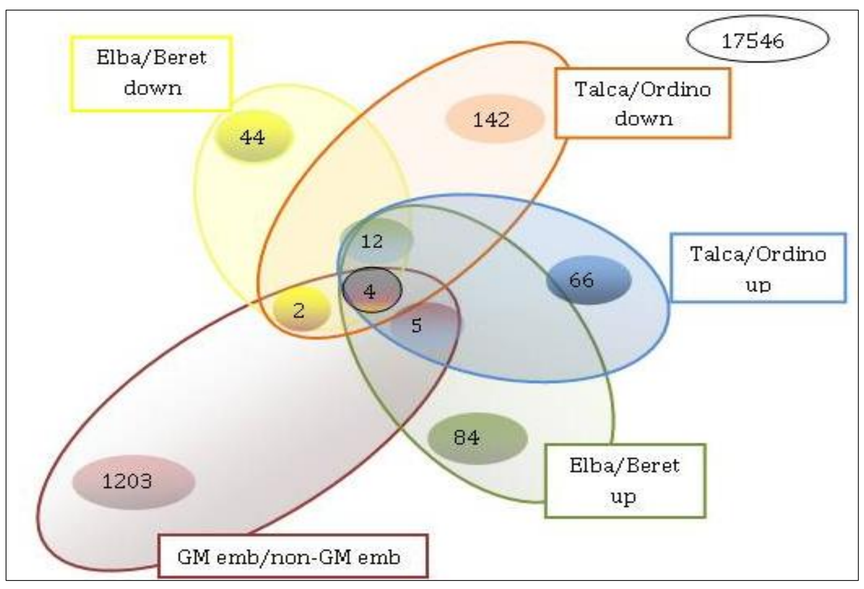

Figure 1. Venn diagram of varieties/tissue-specific differentially expressed protein-coding genes (mRNAs).

Transcript ID	Fold change Talca/Ordino	Fold change Elba/Beret	Fold change HET/HOM N GM	Sequence	Putative target
zma-miR160a-5p	**0,02**	**0,4**	**2,1**	UCCAAAGGGAUCGCAUUGAUCU	**Auxin response factor 22-like**
zma-miR156d-5p	**0,1**	**1,4**	**3,1**	UGACAGAAGAGAGUGAGCAC	**SBP transcription factor13**
zma-miR164f-5p	**0,0**	**0,8**	**3,7**	UGGAGAAGCAGGGCACGUGCU	**Wound responsive protein**
zma-miR171c-3p	**0,0**	**0,5**	**3,1**	UGACUGAGCCGUGCCAAUAUC	**Scarecrow-like protein 27-like**
zma-miR444a	**0,4**	**0,9**	**4,2**	UGCAGUUGUUGUCUCAAGCUU	**MADS-box transcription factor 27-like**
zma-miR171d-3p	**0,0**	**0,6**	**2,5**	UGAUUGAGCCGUGCCAAUAUC	**GRAS transcription factor (GRAS71)**

Table 1. Example of miRNAs sequences (2-fold change), putative targets and plant tissues **where the diferences are observed.**

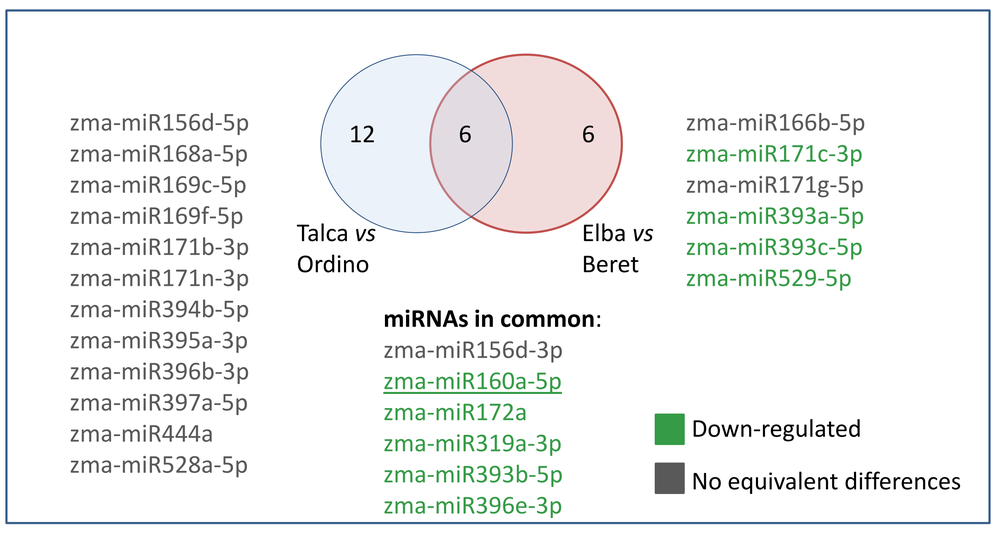

Figure 2. Venn diagram with differentially regulated miRNAs for the transgenic leaves when compared with their near-isogenic non transgenic.

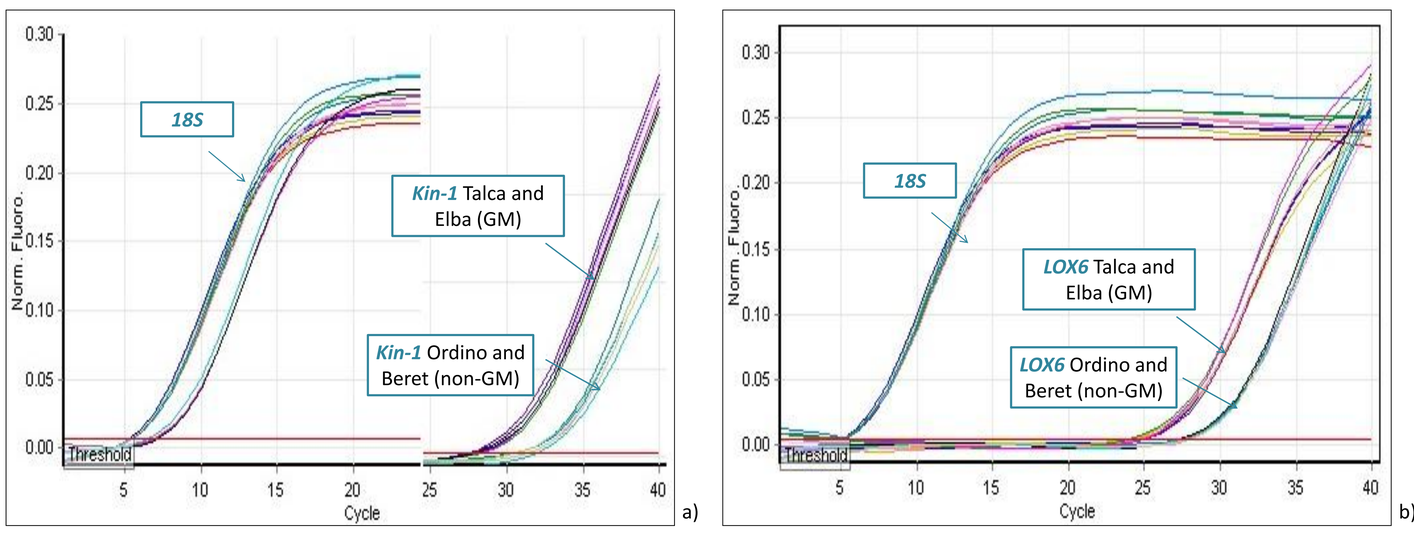

Figure 3 a) and b) Amplification curves for the reference gene (18S) and for two studied genes, *Knotted-1-induced-1* (*Kin-1*) and *Lipoxygenase6* (*LOX6*) for two GM varieties (Talca and Elba) and their near-isogenic non-GM (Ordino and Beret).

Conclusions

- Transcriptomes analyses revealed differences between GM and non-GM varieties correlated with the natural variability but also with the transgene presence;
- In leaves, genes with a consistent differential expression made up to 19 out of which 11 were up-regulated, 3 down-regulated and 5 inversely expressed;
- In embryos the variations made up to 1217 being 723 were up-regulated and 494 were down-regulated. Only 12 genes were common to leaves;
- The predicted target genes of the differentially expressed miRNAs are different from the differentially mRNA obtained in the microarrays, suggesting that more complex relations are expected. Specifically, we focus on:
  - zma-miR160a-5p which regulates genes involved in the auxin signalling pathway, including the *auxin response factor 22-like* and is **down-regulated in our GM leaves**;
  - *ZmLOX6* gene which is known as predominantly expressing in leaves, absent in embryos and regulated by phytohormones and pathogen infections and is **up-regulated in our GM leaves**.
- Based on the existing knowledge concerning *ZmLOX6* up-regulation by jasmonic acid, we speculate that GM maize varieties are less susceptible to pathogen infections.

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